IV-Cell™ Cytogenetics Culture Media

RUO Product Information Packet

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Ver:2402

Contents

Product Overview

IV-Cell™ universal cell culture medium is a Research Use Only (RUO) proprietary culture medium that enables simultaneous culturing of all four (4) hematopoietic cell lineages to solve the problem of selective culturing. IV-Cell™ has been optimized for bone marrow and peripheral blood culturing for in-vitro cytogenetic analysis of hematologic disease, and includes all the necessary components to stimulate the growth of:

1. Myeloid cells
2. T-Lymphocytes
3. B-Lymphocytes
4. Plasma Cells

Media Contents:

- Serum protein
- Media base
- Growth hormones
- Enhancers
- B-Cell mitogens
- T-Cell mitogens
- Plasma-Cell mitogens

Mode of Action: IV-Cell™ contains multiple RS Oligos, each of which was designed to activate a specific cell lineage. These RS Oligos work simultaneously to modulate the interaction of the mitogens with only the specific cells for which they are intended.

Key Product Advantages:

✓ Clinical benefit – culturing of all four cell lineages simultaneously
✓ Independent from carbon dioxide
✓ All-in-one media requires no chemical mixing
✓ Fewer SKUs simplifies inventory management, product ordering, and QC testing
✓ Improved performance of plasma cell stimulation
✓ Higher band resolution reduces karyotyping tech-time

Intended Use

For in-vitro diagnostic use only. Research Use Only (RUO)

Important Information

- Product may be received frozen or cold.
- IV-Cell™ should be stored at 2-8°C and protected from direct light to avoid stimulation of mitogens.
- Allow media to come to room temperature (protected from light) before use.
- Media can be aliquoted directly from 2-8°C storage - as needed, or all at once.
- If the anticipated volume does not require use of entire contents of the bottle, remaining media can either be:
  - Left in the original container, stored at 2-8°C, and consumed within 1 month of opening or before expiration date (whichever comes first)
  - IV-Cell shelf life is 6 months from the production date

Bone marrow or leukemic peripheral blood received should be cultured in duplicate according to the indication for the particular study requested. This provides the best opportunity to verify true mosaicism and supplies back up cultures in the event of failures due to contamination, technical error, and/or other problems.

Aliquot Instructions

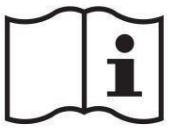
To aliquot IV-Cell™, please follow the steps below:

- Prepare the sterile biological hood by wiping the surface thoroughly with 70% ethyl alcohol.
- Remove IV-Cell™ bottle from 4°C storage and wipe the external surface of the bottle with 70% ethyl alcohol.
- Prepare the desired number of sterile 15 mL tubes for aliquoting media.
- Using a serological pipette, in a sterile manner, aliquot 5 mL of IV-Cell™ media to each sterile 15 mL tube.
  NOTE: To maintain sterility, it is recommended that the serological pipette tip is changed every 10 aliquots. It is also recommended that when the pipette is changed, that the media bottle is recapped and inverted to assure the media remains uniformly mixed throughout allocation.
- Once the aliquot is completed, assure that all sterile 15 mL tubes are closed tightly.
- The new aliquots should be stored at 4°C and protected from direct light.
- Media expiration is one year from the date of manufacture or 1 month from the date first opened.

Safety Information

- Read the material Safety Data Sheets (SDS) and follow recommended handling instructions for this product.
- Wear appropriate protective personal protective equipment (eyewear, clothing & gloves).
- Handle in accordance with established bio-safety practices.
- The material safety data sheets can be downloaded from the following link: www.precipiodx.com/ivcell.html

Product	IV-Cell Culture Media
Catalog Number	IVC2-100, ICV2-500
Amount	100-500 mL
Storage	4°C (protect from light)
Shelf Life*	6 months from manufacture, 1 month once opened
** Shelf Life is determined from date of manufacturing Do not use beyond the expiration date.*	



Recommended Procedures

Culture Set-Up

1.1. Allow IV-Cell™ culture media to come to room temperature (protected from light)
1.2. Once media is at room temperature, store inside the bio-hood to avoid contamination
1.3. Set up all cultures **in the 15 mL tubes in which the media was aliquoted**
    1.3.1. Each culture is to be inoculated up to 500µl (see reference table below)
1.4. Place closed 15 mL tube (with media and sample) in incubator at approximately a 30-45° angle
1.5. Follow tables below for inoculation volumes and incubation times per cell type of interest

Table 1: Specimen Volume Requirements

Cell Count (1:100 Dilution) (x10^6 cells/ml)	Specimen volume (µl)/ 10ml medium	Specimen volume (µl)/ 5ml medium
10	1000	500
20	700	400
30	500	300
40	300	200
50	300	150
60	200	100
70	200	100
80	200	75
90	150	50
>/=100	150	50

Table 2: Culture Incubation Periods

Cell Type	Incubation Period (Culture 1)	Incubation Period (Culture 2)
Myeloid Cells	18-24 hours	32-48 hours
T-Lymphocytes	18-24 hours	72 hours
B-Lymphocytes	18-24 hours	72-96 hours
Plasma Cells	18-24 hours	96-120 hours

IMPORTANT:
In order to benefit from the clinical advantages IV-Cell™ offers, obtaining the flow cytometry and morphology results prior to determining the incubation period will help the cytogeneticist in selecting the cell lineage clinically relevant to the case.

Culture Harvesting

*Harvesting Note: Incubation times indicated are for chemical incubation. When working with numerous samples, transfer time must be accounted for. Example: When the 30-minute incubation period [Step 1.11] is over, tubes should be **in** HANABI so the next process can start as close to the 30-minute mark as possible.*

2.1. Have enough hypotonic solution (0.075M KCl) at 37°C for the cultures to be harvested that day
2.2. Prepare fresh fixative (3 parts Methanol: 1part Glacial Acetic Acid) at room temperature for the cultures to be harvested that day
2.3. Add 75µl EB (Ethidium Bromide) or CRA (Chromosome Resolution Additive) (suggested dilution 1mg/ml) to each 5ml culture, mix well by capping and inverting tube
2.4. Incubate at 37°C for 1 hour
2.5. Add 100µl of Colcemid to each 5ml culture, mix well by capping and inverting tube
2.6. Incubate at 37°C for 30 min
2.7. Transfer 15ml tubes with cultures to HANABI for remainder of harvest procedure
2.8. When harvest protocol has completed, remove tubes from HANABI and evaluate supernatant of all samples.
NOTE: It is recommended that caps used before HANABI are discarded and fresh caps be placed on the tubes after HANABI for the sake of streamlining. Matching caps to tubes becomes time-consuming at high volumes.
2.9. If supernatant is clear, proceed to step 1.16
2.10. If supernatant is not clear, perform additional wash(es) until it is:
2.10.1. Aspirate the supernatant carefully, leaving around 0.5 ml supernatant above the pellet
2.10.2. Gently re-suspend the pellet by tapping the side of the tube
2.10.3. Add fixative up to 5ml (or 3ml if pellet is very small) and gently invert tube to mix
2.10.4. Centrifuge for 10 min at 1000 rpm
2.10.5. If supernatant is not clear, repeat steps 1.15.1-1.15.5
2.11. Aspirate the supernatant carefully, leaving around 0.5 mL supernatant above the pellet
2.12. It is recommended to leave the pellet in the refrigerator for 30 min before dropping for higher quality banding

Slide Preparation

3.1. Re-suspend pellet in a small volume of carnoy fixative (0.5 - 1 mL). The pellet should be white and the suspension should appear cloudy after re- suspension
3.1.1. *Evaluate solution made:* If pellet is too concentrated, more fixative can be added. If the pellet is too dilute, sample can be spun down and re-suspend in a smaller volume.
3.2. Using a new transfer pipette for each culture tube, drop slides from one case at the time.
3.3. Dip a clean slide in a 250ml beaker of water combined with a capful of 200 proof ethanol.
3.4. Remove slide from the beaker and blot excess liquid from edges
3.5. Hold the slide at a slight (5-15°) angle and drop ~3 drops of the cell suspension down the length of the slide
3.6. Blot the edges of the slide to remove excess liquid
3.7. Rinse the slide with fix by gently running fix down one of the long edges of the slide and allowing the solution to wash over the front of the slide
3.8. Blot the edges of the slide to remove excess liquid and wipe off the back with a paper towel
3.9. Dry slides
Note: optimal conditions are 28°C and 30% relative humidity; this can be achieved with a humidity chamber or by controlling temperature and humidity at the bench top.
3.10. Age the slides in the oven at 90°C for 90 minutes.

Slide Staining

Staining Note: For high volumes, alternate staining methods may be used. Multiply volumes by appropriate factor to fill containers.

4.1. Set up 4 staining jars and proceed with staining slides per the table below
4.2. Let slides air dry at an angle or use bibulous paper
4.3. Store slides at room temperature until ready to scan

Table 3: Slide Staining

Jar #	Contents	Procedure
Jar 1	Mixture of pH 7.0 Buffer & Trypsin Solution	Submerge slides for 40 sec (adjust time as needed)
Jar 2	50 mL Balanced Salt pH 7.0	Dip slides twice
Jar 3	Mixture of pH 6.8 Buffer & Wright's Stain (or equivalent)	Submerge slides for 1 min 20 sec (adjust time as needed)
Jar 4	50 mL Distilled water	Dip slides twice

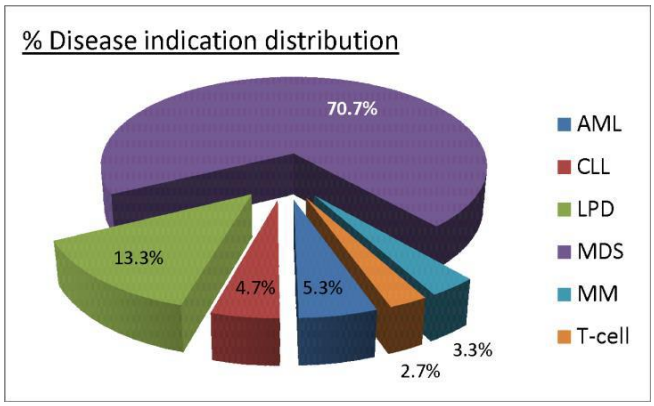
Performance Results (Internal)

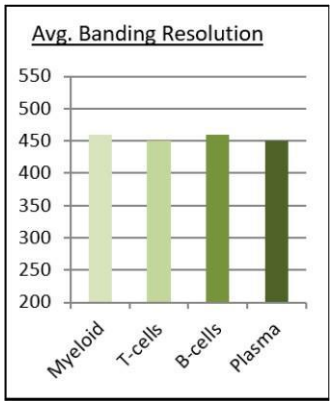
Overview:
IV-Cell™ Media was first used clinically at Precipio's laboratory in April 2017. The following data presents Precipio's internal results from the application of IV-Cell™ media to Karyotyping cases within Precipio's department of cytogenetics.

Method:
150 Bone Marrow Biopsy cases were randomly selected where Karyotyping was conducted. The results depict the disease state distribution and the banding resolution received by using IV-Cell™ media.

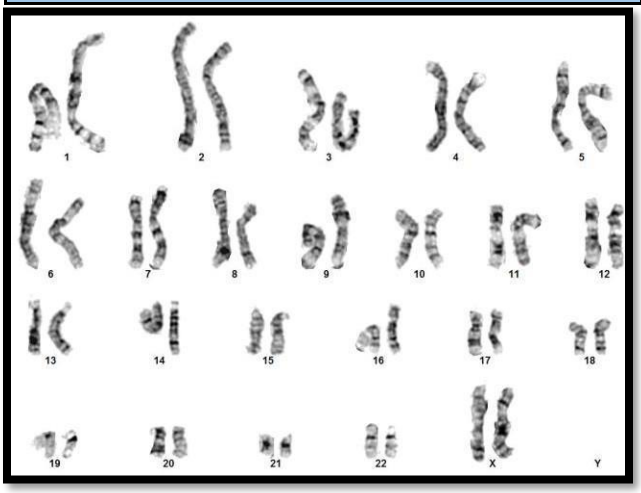
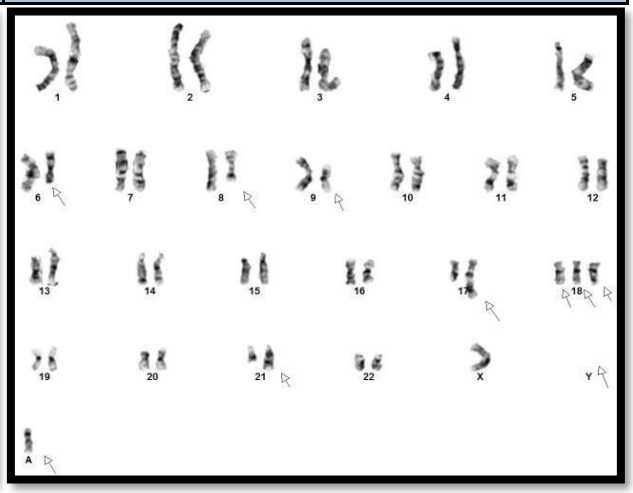
Results:
As shown in the images on this page, IV-Cell™ media provided consistently high results as measured by the banding resolution. Average band resolution is 460, with some chromosomes reaching banding resolution of above 500.

Disease	#	%
AML	8	5.3
CLL	7	4.7
LPD	20	13.3
MDS	106	70.7
MM	5	3.3
T-Cell	4	2.7
	150	**100**





Summary Table	
Number of cases	150
Avg. Banding Resolution	460

High Resolution Normal Karyotype (550 band resolution)	High Resolution Abnormal Karyotype (500 band resolution)
	

Explanation of Symbols and Warnings

			
Caution, consult accompanying documents	*In vitro* diagnostic medical device	Sterilized using aseptic processing techniques	Keep away from light
			
Use By:	Catalog number	Manufacturer	Batch Code
			
European Community	Consult instructions for use	Temperature Limitation	

Each manufactured lot of IV-Cell™ is performance-tested on primary normal bone marrow cells to ensure product performance for in vitro diagnostic use for this application.

Each clinician/scientist must make an independent judgment on whether this medium is suitable for use in In Vitro Diagnostic applications conducted in their laboratory. Precipio does not guarantee the successful outcome of any diagnostic testing based solely on the use of IV-Cell™ medium. Precipio's contribution to these procedures is simply at the step of providing a culture or handling medium for these procedures.

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